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Enantiopure Oxazoloisoindolinones: Promising Small Molecules for p53-based Therapy with Potential Anticancer Properties

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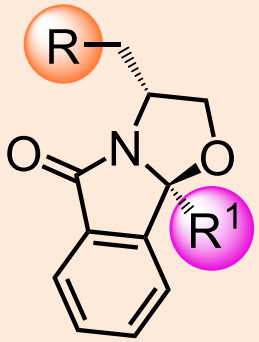
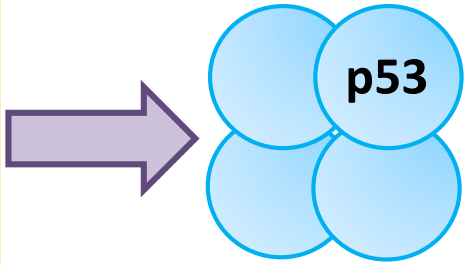
OXAZOLOISOINDOLINONES





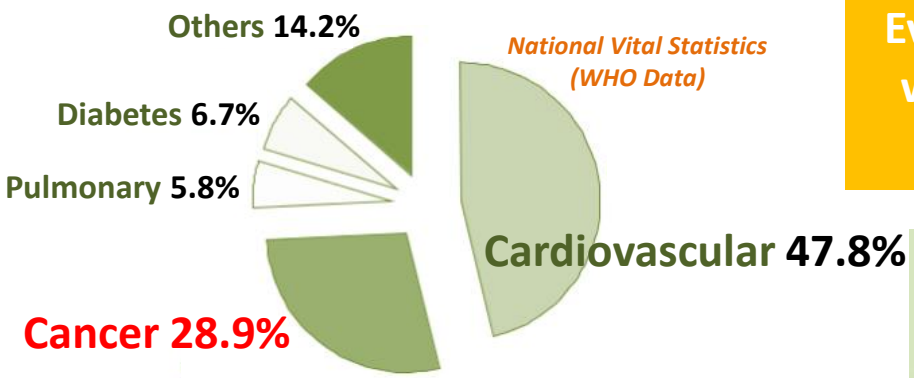
- ✓ selectivity
- ✓ potent antitumor activity
- ✓ no toxic effects

Abstract: The tumor protein p53 is a widely-studied therapeutic target in cancer treatment, as this transcription factor is inactivated in all types of human cancers. In 50% of malignancies, p53 is expressed in its *wild-type* form and generally inhibited by two major negative regulators, MDM2 and MDMX. In the remaining 50% of cases, p53 is inactivated by mutations principally on its DNA-binding site, thus not exercising its regulatory function. In the last years, our research group has been involved in the synthesis of potential p53 reactivators. Starting from the enantiopure aminoalcohol tryptophanol, we have recently developed several small molecules that reactivate p53. Here we present our most updated results on the development of a chemical library of tryptophanol-derived oxazoloisoindolinones. This class of compounds is accessed by cyclocondensation reaction of enantiopure forms of tryptophanol and several achiral oxoacids. In this synthetic approach, the chiral inductor is responsible for the stereo-outcome of the final product and it is part of the main skeleton of the bioactive molecules. From this work bicyclic lactams SLMP53-1 and DIMP53-1 were identified as the most promising hits. Further hit-to-lead optimization is ongoing, and assessment of the antiproliferative activity of the optimized oxazoloisoindolinones against four different cancer cells lines highlights that this chemical family displays potent antitumor activity towards p53 with no apparent toxic effects.

Keywords: Cancer, p53, Tryptophanol, Enantiopure Drugs, Antitumor activity

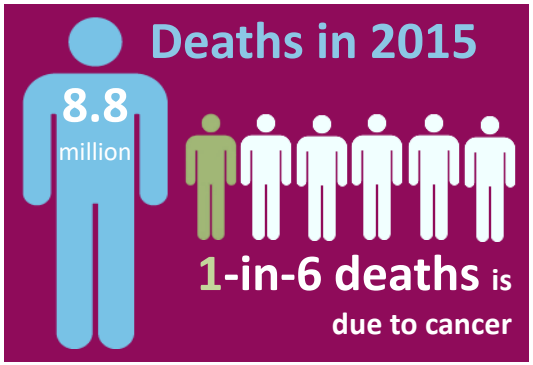
Introduction - Cancer in facts

Cancer is a group of diseases that can affect any part of the body *via* an uncontrolled and anomalous cellular proliferation

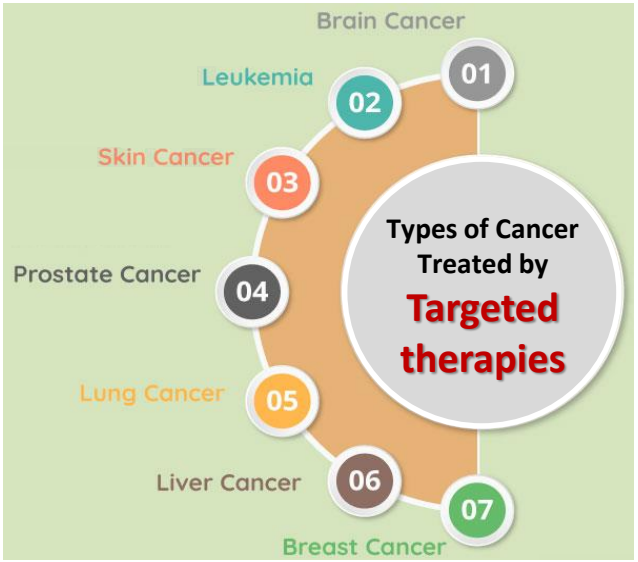


Every year **14 million** people world-wide hear the words:

"You have cancer"



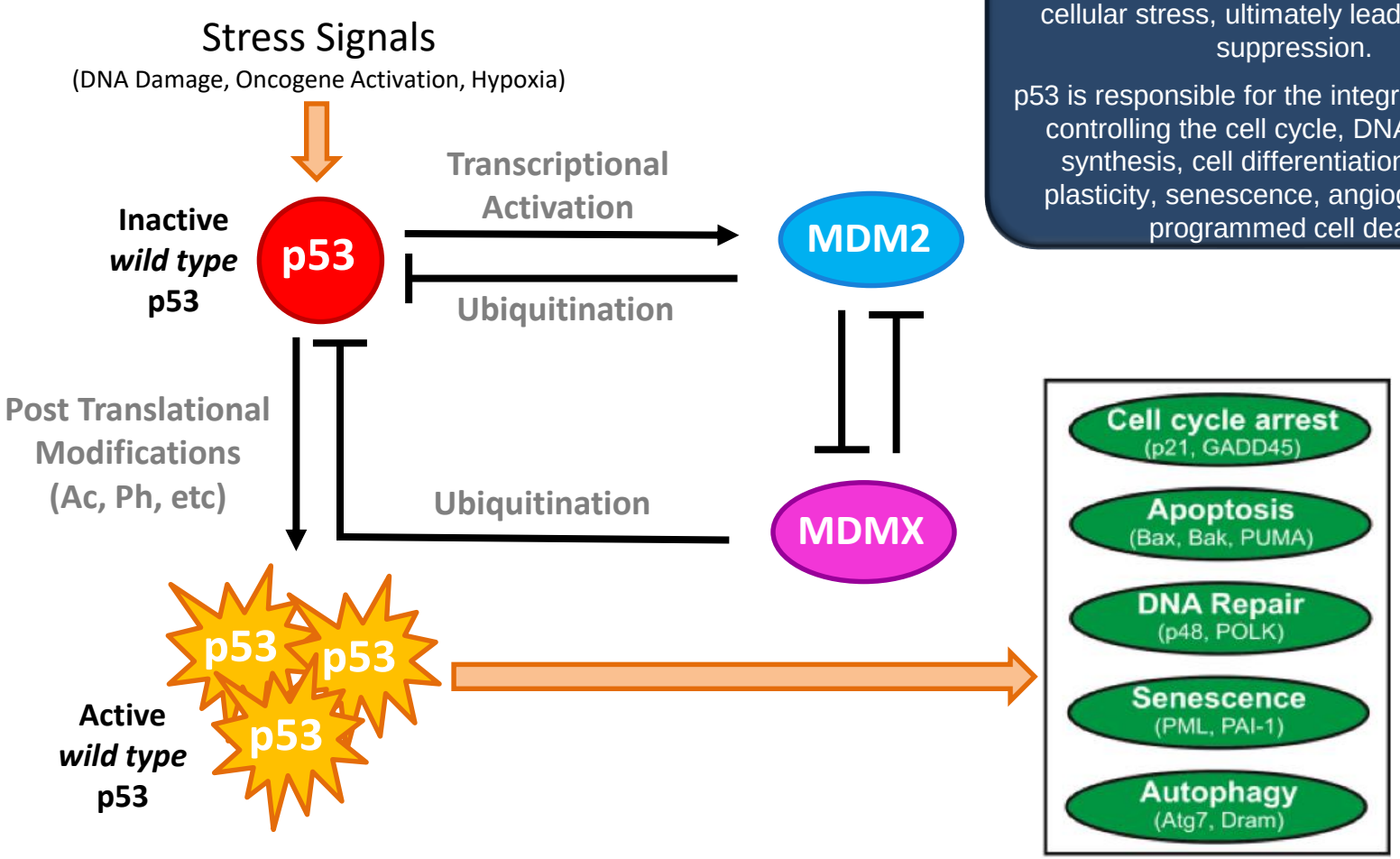
2nd leading cause of death globally after cardiovascular diseases



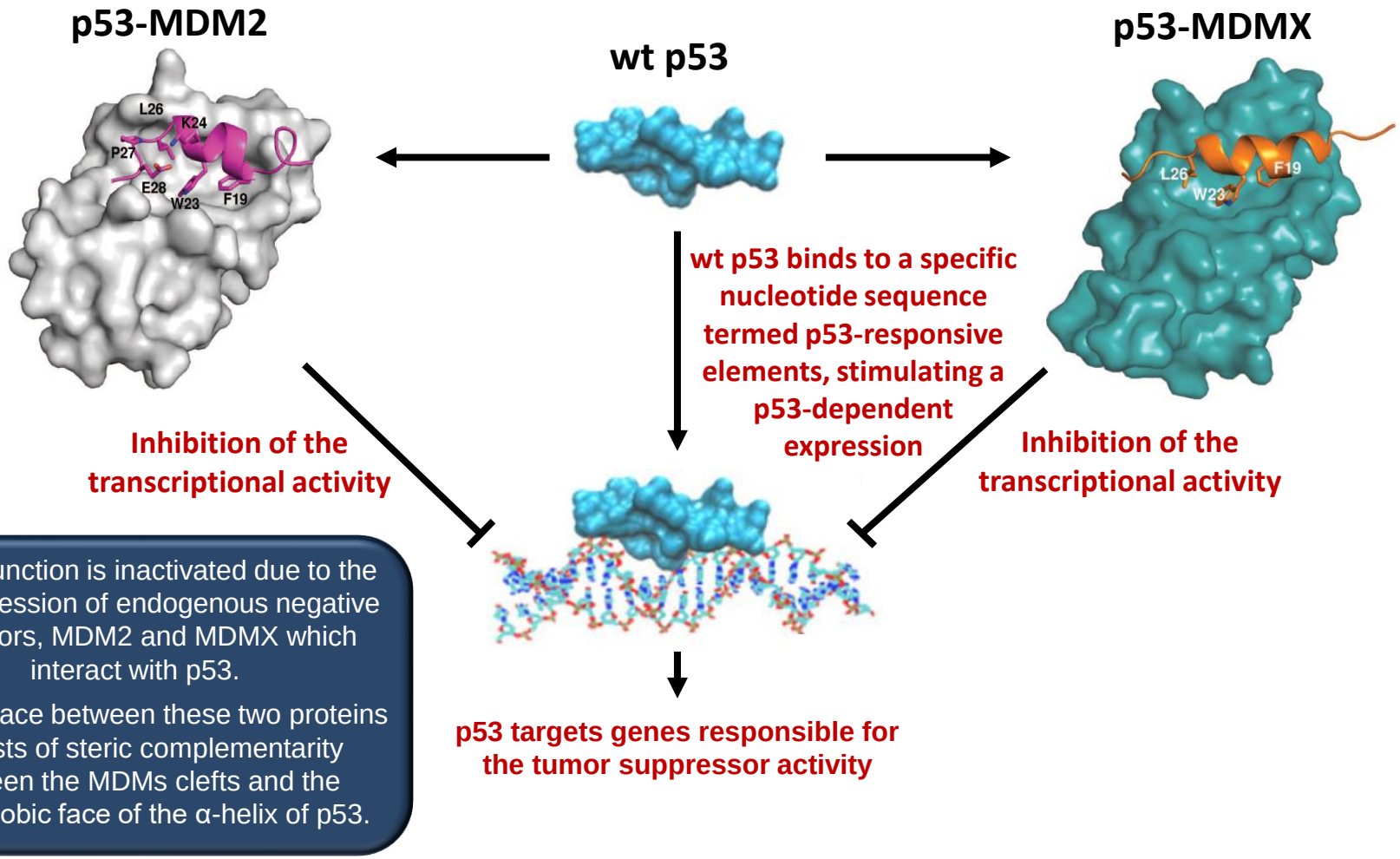
Introduction - Role of p53 in Cancer

The p53 signaling pathway is activated under cellular stress, ultimately leading to tumor suppression.

p53 is responsible for the integrity of the cells, controlling the cell cycle, DNA repair and synthesis, cell differentiation, genomic plasticity, senescence, angiogenesis and programmed cell death.



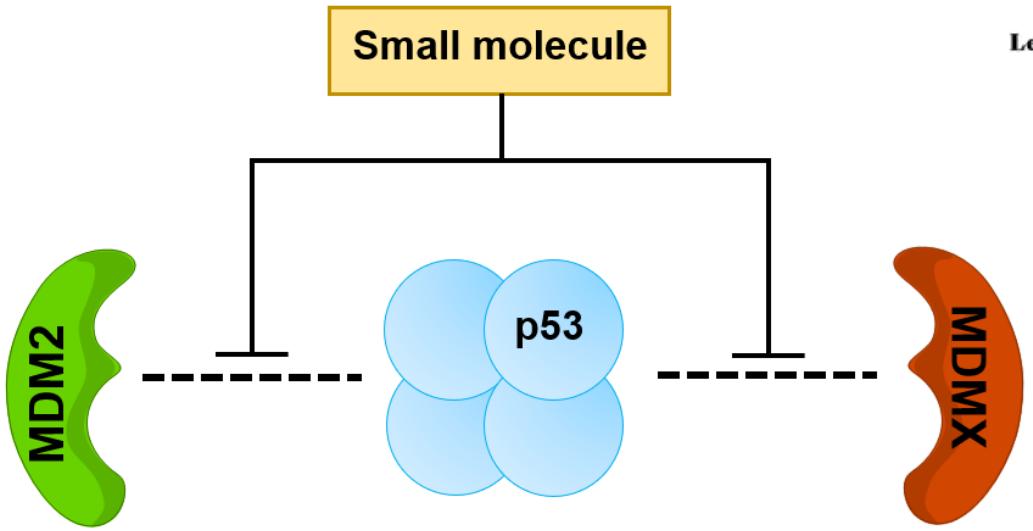
Introduction - *wild type* p53, MDM2 and MDMX

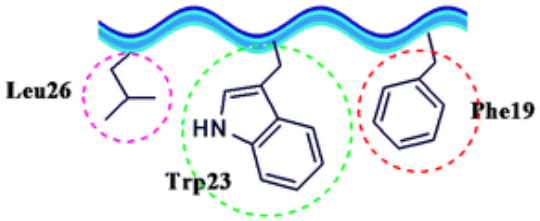


wt p53 function is inactivated due to the overexpression of endogenous negative regulators, MDM2 and MDMX which interact with p53.

The interface between these two proteins consists of steric complementarity between the MDMs clefts and the hydrophobic face of the α-helix of p53.

Introduction - Reactivation of *wild type* p53





There are 3 key hydrophobic residues in p53 responsible for the interaction of p53-MDM2: Phe19, Trp23 and Leu26

The p53 activity can be restored using different strategies, depending on the p53 status: in case of wt p53, reactivation is carried out by inhibition of its main negative regulators

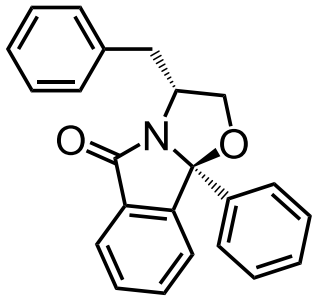
- **p53-MDM2 inhibitors**

Only 8 candidates in clinical trials (2 discontinued)

- **NO Dual p53-MDM2/X inhibitors** in clinical trials

Introduction - Hit compounds developed by Santos's team

The first oxazoloisoindolinone developed was compound 3a, a bicyclic lactam derived from the aminoalcohol phenylalaninol

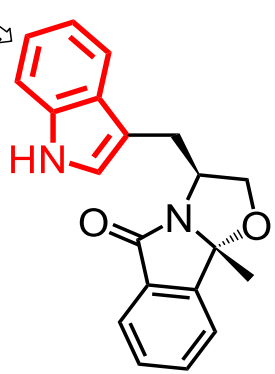


3a

p53-MDM2 inhibitor

Soares J. *et al.,* Eur. J. Pharm. Sci., **2015**, 66, 138

indole moiety prioritised

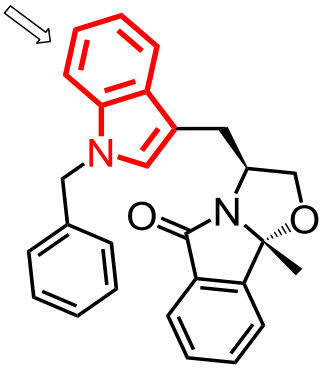


SLMP53-1

***wt* and *mut* p53 reactivator**

Soares J. *et al.,* Oncotarget, **2016**, 7, 4326

indole moiety prioritised

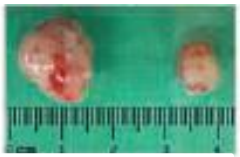


DIMP53-1

p53-MDM2/X dual inhibitor

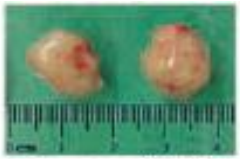
Soares J. *et al.,* Mol. Oncol., **2017**, 11(6), 612

HCT116p53$^{+/+}$



Control SLMP53-1

HCT116p53$^{-/-}$



Control SLMP53-1

SLMP53-1 potently suppresses the growth of *wt/mut* p53-expressing tumors, but not of p53-null tumors, in xenograft mice models

Patent

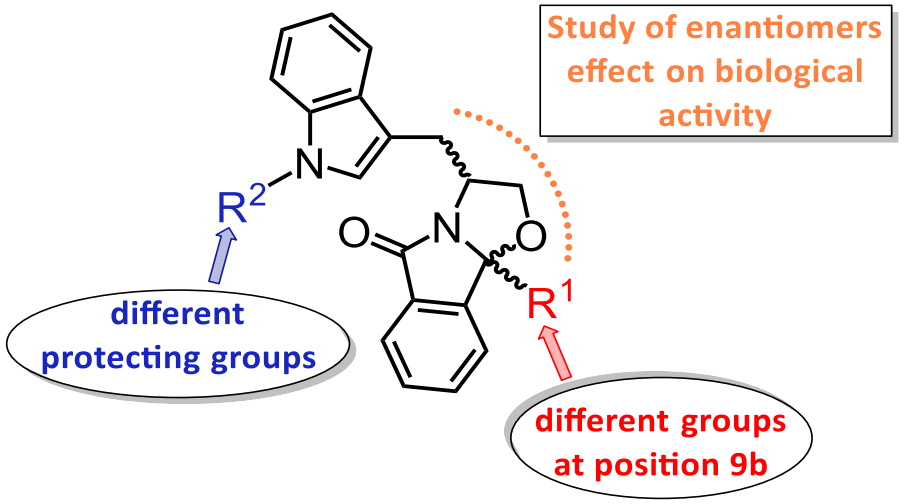
Saraiva L., Santos M.M.M., *et al.*, WO2014207688**, 2014**

Results and discussion - Ongoing Hit-to-Lead Optimization

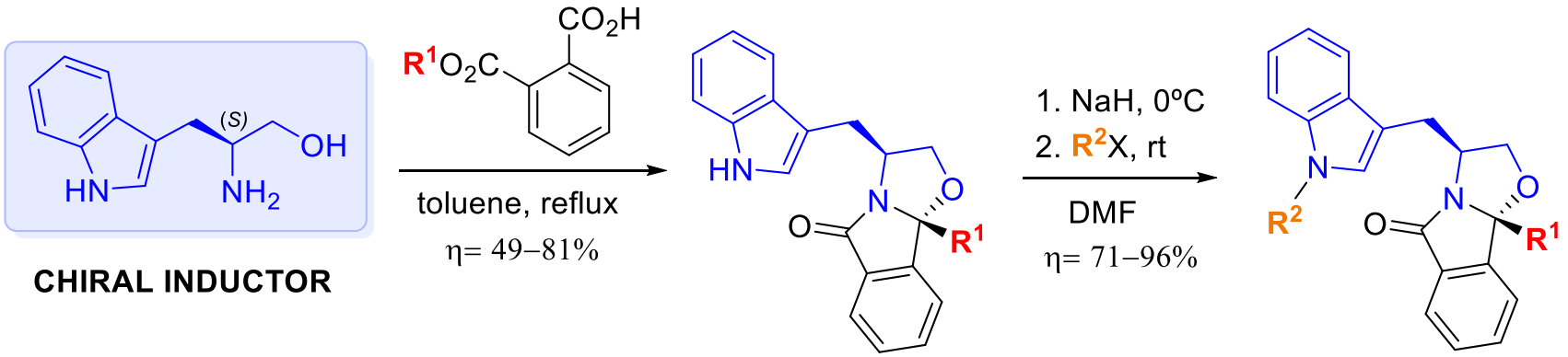
Hit Identification → **Lead Optimization**

SLMP53-1

DIMP53-1



Results and discussion - Synthesis of oxazoloisoindolinones



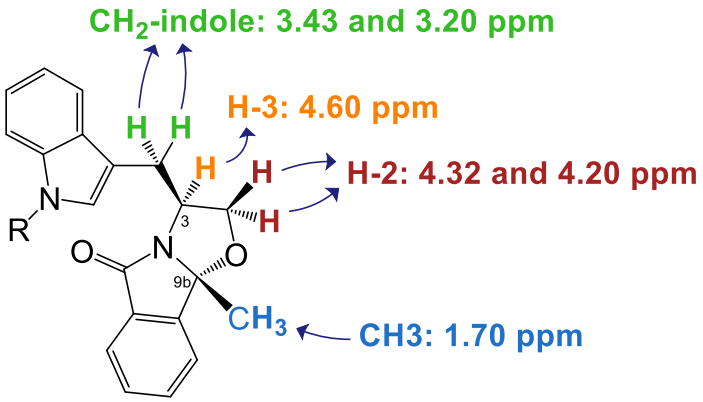
Oxazoloisoindolinones are accessed by cyclocondensation reaction of enantiopure forms of tryptophanol and several achiral oxoacids. In this synthetic approach, the chiral inductor is responsible for the stereo-outcome of the final product and it is part of the main skeleton of the bioactive molecules.

R^2=Me; Et; Pr; Ac; Bn; Bz; Ts.

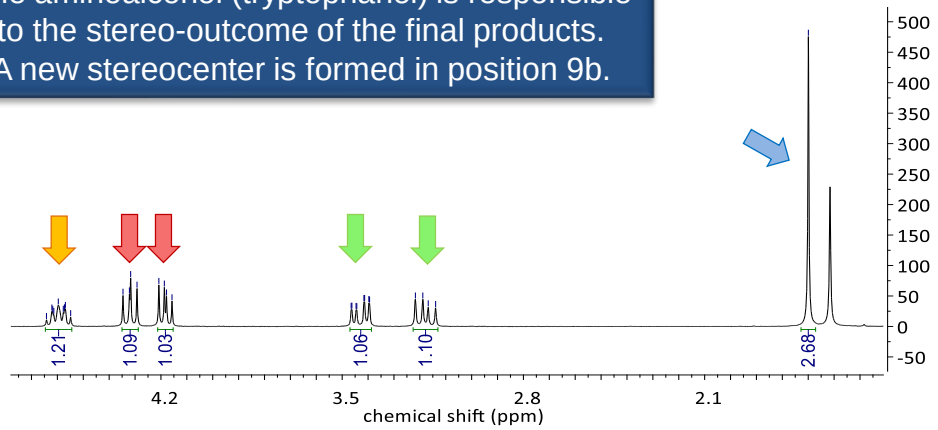
R^1=Me; Ph; *p*-F-Ph; *p*-Cl-Ph; *p*-CH_3-Ph; *p*-Cl,m-NO_2-Ph.

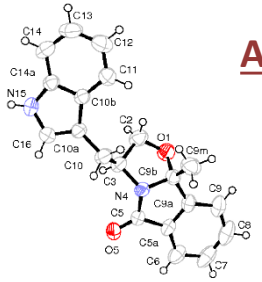
35 compounds synthesized

Results and discussion - NMR characterization



The aminoalcohol (tryptophanol) is responsible to the stereo-outcome of the final products. A new stereocenter is formed in position 9b.





Absolute configuration

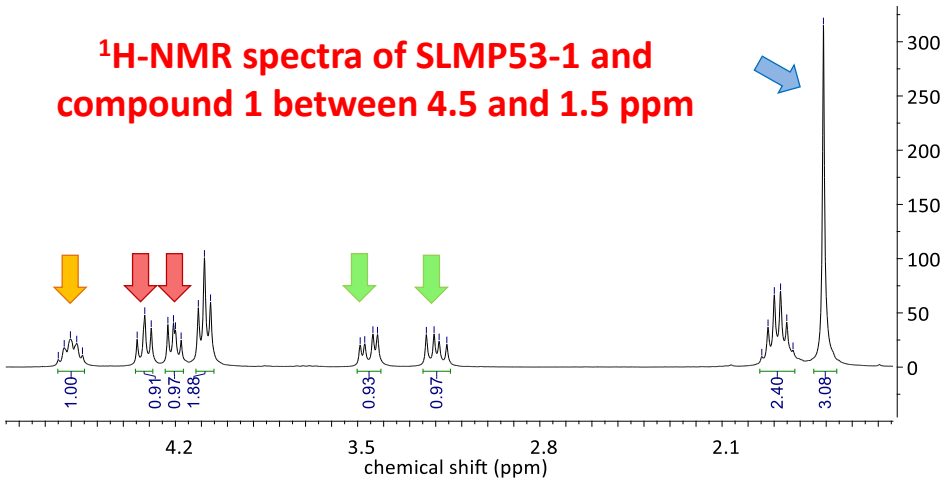
established by

- ✓ X-ray crystallographic analysis of SLMP53-1
- ✓ ^{13}C-NMR analysis

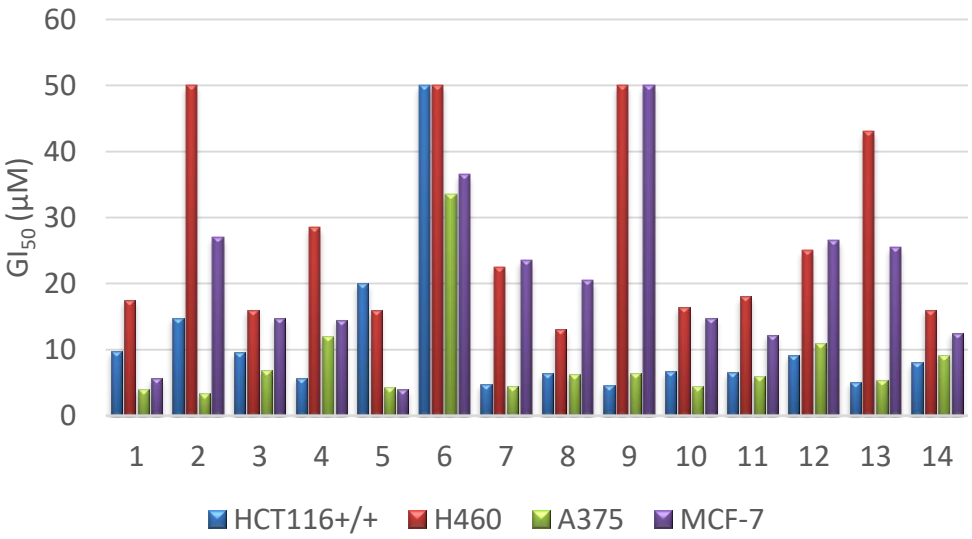
compound	C-9b	C-2	C-3	CH_2-indole
SLMP53-1	98.9	74.6	56.0	30.8
1	99.2	74.7	56.2	30.6

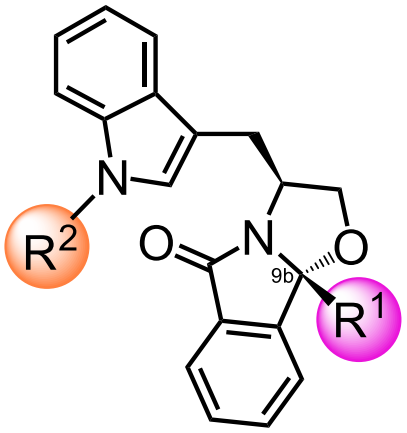
Chemical shifts expressed in ppm.

^{1}H-NMR spectra of SLMP53-1 and compound 1 between 4.5 and 1.5 ppm



Results and discussion - Biological evaluation towards *wt* p53





Assessment of the **antiproliferative activity** of the optimized oxazoloisoindolinones against:

- ✓ **Human colon carcinoma, HCT116**
- ✓ **Human lung carcinoma, NCI-H460 cell line**
- ✓ **Human malignant melanoma, A375**
- ✓ **Human breast adenocarcinoma, MCF-7**

highlights that most of the bicyclic lactams composing this chemical family displays **potent antitumor activity** once the derivatives are assayed in A375 cell line.

Structure-activity relationship studies

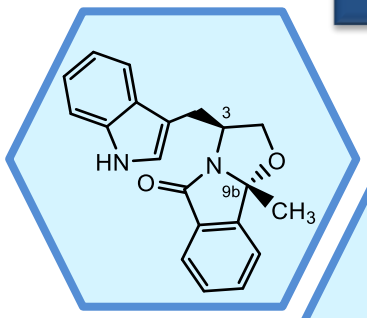
➢(*S*)-tryptophanol-derived bicyclic lactams are more active than the corresponding enantiomers.

➢Introduction of aromatic groups in position 9b and the presence of bulky and electron-withdrawing groups on the indole nitrogen improve the activity.

Results and discussion - *in vitro* Stability studies

MICROSOMAL STABILITY

SLMP53-1 and DIMP53-1 were selected to assess the *in vitro* stability of the chemical family in human microsomes.

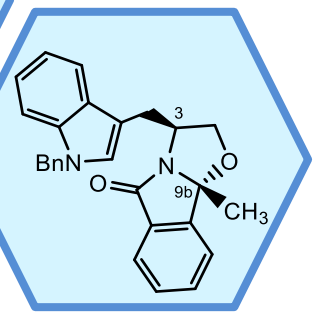


DIMP53-1

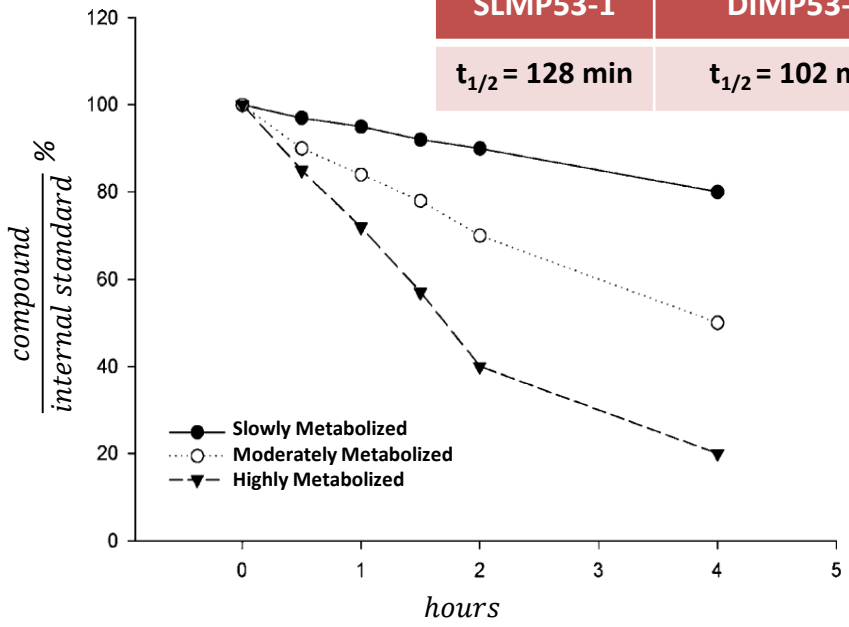
After 2 hours 69% went under metabolic Phase I chemical modifications

SLMP53-1

After 2 hours 58% went under metabolic Phase I chemical modifications



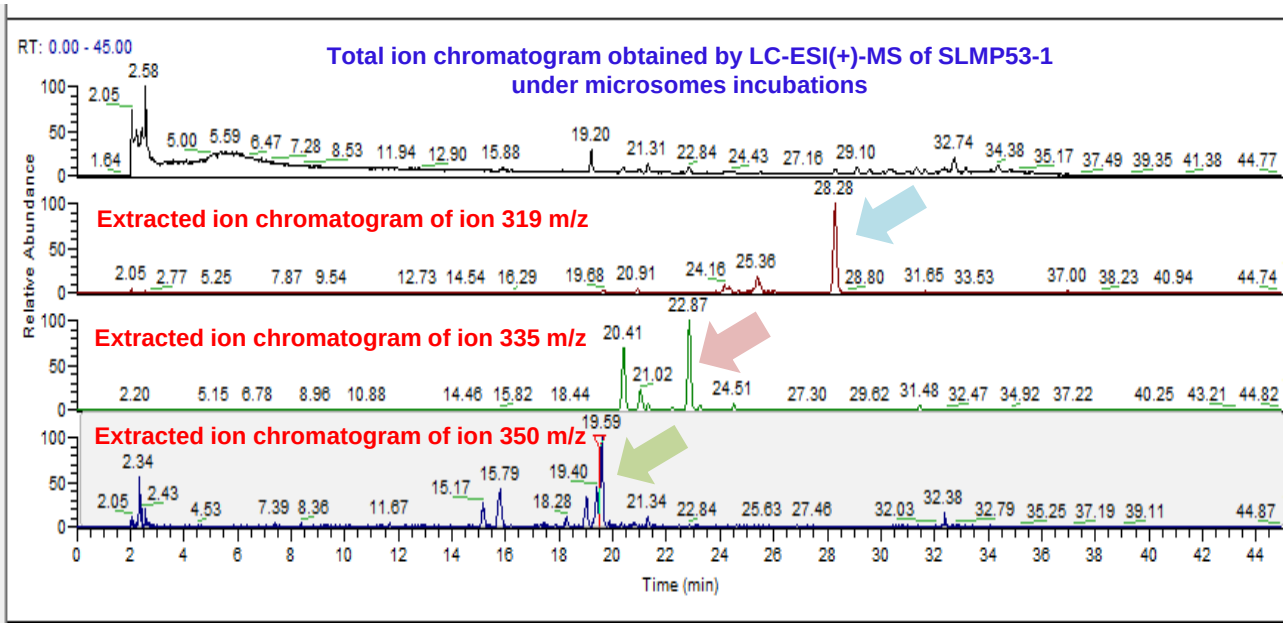
SLMP53-1	DIMP53-1
$t_{1/2}$ = 128 min	$t_{1/2}$ = 102 min

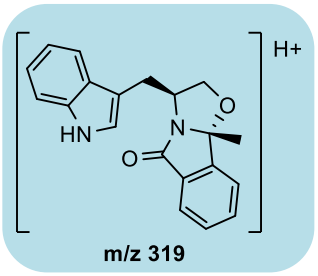


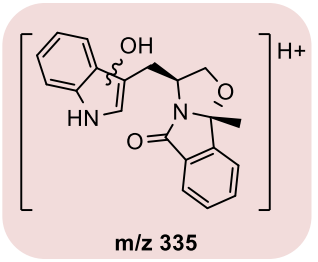
Yan Z., Caldwell G.W., Methods in Pharmacology and Toxicology - Optimization in Drug Discovery: *in vitro* methods., **2014**, 10: 151-162.

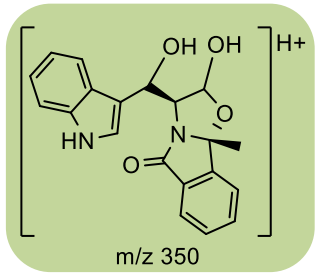
Results and discussion - *in vitro* Stability studies

SCREENING OF PHASE I METABOLITES – SLMP53-1





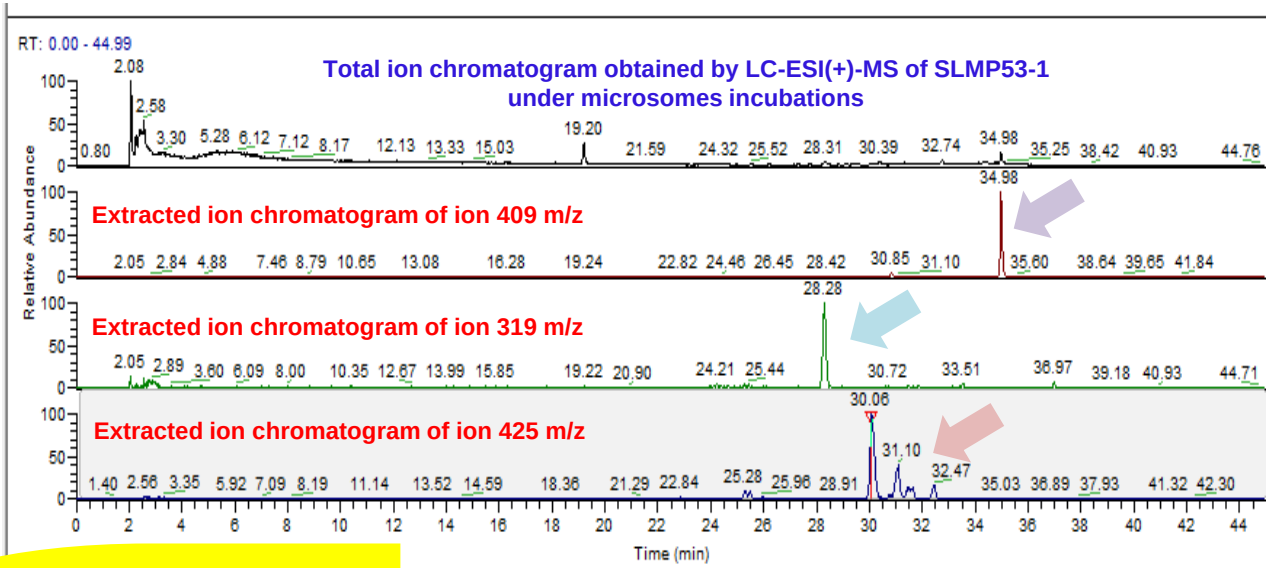


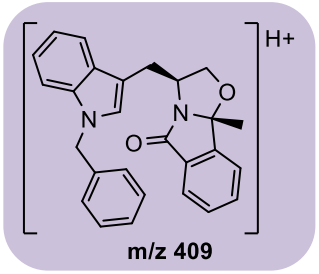


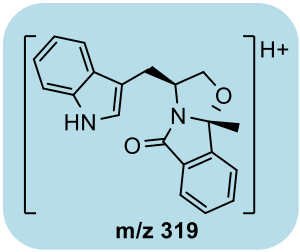
- ✓ 2 major and 1 minor monohydroxylated metabolites found.
- ✓ 1 major and 4 minor dihydroxylated metabolites found.

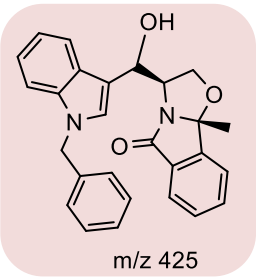
Results and discussion - *in vitro* Stability studies

SCREENING OF PHASE I METABOLITES – DIMP53-1









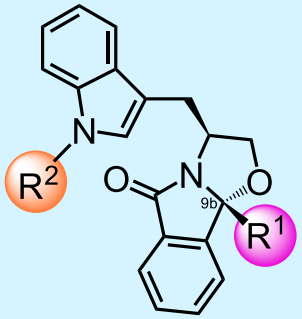
No di-hydroxylated metabolites observed for DIMP53-1

- ✓ DIMP53-1 is metabolized into SLMP53-1
- ✓ 1 major and 2 minor monohydroxylated metabolites found.

Conclusions

Hit-to-lead optimization of SLMP53-1

- Library of **35** bicyclic lactams obtained with good to excellent yields between **71** and **96%**



Evaluation of the antitumoral bioactivity of the lead generation

- most of the bicyclic lactams composing this chemical family displays **potent antitumor activity** once the derivatives are assayed in A375 cell line.

Evaluation of the stability studies of SLMP53-1 and DIMP53-1

In human microsomes: moderate stability

- For **SLMP53-1**: **2** major mono-hydroxylated metabolites found
- For **DIMP53-1**: **1** major mono-hydroxylated metabolite found

Acknowledgments

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